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THE POSSIBILITY OF TYPHOID INFECTION THROUGH VEGETABLES *

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Vegetables, grown on land fertilized with night soil and eaten raw, have long been regarded as a possible source of typhoid infection. A few cases are on record which have been attributed to this cause.

Warry¹ reported in 1903 an outbreak of 110 cases occurring in Hackney, a suburb of London, considered due to the eating of watercress grown in beds fertilized with sewage. Pixley² records 2 cases of typhoid from eating uncooked rhubarb, which was grown in soil known to have been fertilized with typhoid excreta. Morse³ attributed 49 cases among the inmates of an insane asylum to the eating of celery. In this instance, there had been typhoid fever in the institution some months previous and the celery beds had received the hospital sewage. The disease developed soon after the celery came into use.

An outbreak occurred in Philadelphia apparently due to contaminated watercress served at a wedding breakfast June 24, 1913, with 43 guests in attendance. Of the 19 persons who ate watercress sandwiches, 18 were ill with typhoid on July 22. Investigation by the Philadelphia Bureau of Health⁴ showed that the watercress had been secured from a farm where sanitary conditions were quite unsatisfactory. Typhoid bacilli were not isolated from the cress beds, but all the other circumstances of the outbreak afforded strong reason for suspecting the watercress to have been the vehicle of infection.

The explanation for the few successful attempts to trace an epidemic to vegetables is apparent. Ordinarily, such articles of food are distributed among a wide circle of consumers after passing through the hands of several dealers; the difficulty of discovering weeks afterward, what vegetables were eaten and by whom, and the final tracing of them to their source of contamination may baffle the epidemiologist.

In order that vegetables shall serve as a means for disseminating typhoid from contaminated soil, there must be at least 2 positive factors.

The viability of the typhoid bacillus must extend from the time of manuring the beds until the vegetables have been consumed. In the majority of cases, the time would be approximately that required for

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¹ Lancet, 1903, p. 1671.

² New York Med. Jour., 98, 1913.

³ Rep. Bd. Health Mass., 1899, p. 761.

⁴ Engineering News, Aug. 14, 1913.

the growing and marketing of the vegetables, since manuring of the soil usually takes place a short time before the planting or during the growing season.

The typhoid bacillus must also adhere to the plant with sufficient tenacity to withstand the natural precipitation and other factors during the growing season, as well as those of marketing, and must survive the cleaning methods ordinarily employed in the preparing of such food for table use.

The longevity of the typhoid bacillus in soils has been a disputed question. Differences in the reports of observers can be largely accounted for by the artificial conditions used in some experiments, as well as by the faulty methods used in isolation and identification.

The results of the more trustworthy experiments indicate that the longevity of the typhoid bacillus in soil is greater than that required for the production of many common, truck vegetables.

The most definite experimental work on the infection of vegetables grown in typhoid-infected soil has been conducted by Creel,⁵ who reports the isolation of *B. typhosus*, after a period of 25 days, from the leaves and upper stems of lettuce and radishes grown under hot-house conditions in soil contained in glass jars. From plants grown in the open, he isolated the organism in 2 experiments from the leaves and stems after 31 and 10 days, the approximate exposure to direct sunlight being 138 and 84 hours, respectively. In this work, the method used in inoculating the soil in each case was by adding a fecal emulsion mixed with 24-hour agar cultures of *B. typhosus*. To gain further information about the possibility of infection by this means, under approximately natural conditions, and also to compare such results with those under modified conditions, the following experiments have been made on vegetables grown in the hot-house and in the open.

INDOOR EXPERIMENTS

Preliminary experiments were conducted during the winter 1914-1915 under hot-house conditions. Radishes and lettuce were grown in soil contained in large earthen-ware, screened pots. Shortly after the planting of the seeds, inoculation of the soils was made by the addition of suspensions of *B. typhosus*. In some cases, a suspension from agar slants in physiologic salt solution was used; in others, a broth suspension. Two strains were used: One was an old stock culture, desig-

⁵ Pub. Health Rep., 1912, 27, p. 187.

nated as Strain 1; the other, Strain 2, was isolated 21 days previously from fresh feces.

Tests were begun shortly after the appearance of the plants above the surface and repeated at intervals of 3-5 days. The lettuce stems were cut off above the surface, without allowing the leaves to come in contact with the soil; in the case of the radish the entire plant was removed from the soil and the tops cut off. The leaves and stems of the lettuce and the roots of the radishes were then placed in separate shallow pans and washed 2-3 minutes in running water, during which time they were rubbed with the hands, protected by sterile rubber gloves.

The vegetables were then removed and rinsed 2-3 times in sterile water, and finally placed in a sterile mortar and finely macerated in a few cubic centimeters of sterile water. Plates were then made from the final product and the rinse water.

TECHNIC

For plating, freshly prepared Endo's medium was used. I have obtained the best results in the preparation of the medium by using 7 c.c. of a saturated alcoholic solution of basic fuchsin, and decolorizing by the addition of a 10% solution of sodium sulfite, until a faint pink remained on cooling, rather than by adding a definite amount of sodium sulfite.

The plates were arranged in 6-8 Series, poured and allowed to harden with the covers partly removed; allowing 20-30 minutes for the medium to become firm. The fluid from the macerated plants and their rinse water was then transferred with sterile pipettes to the centers of the first 4-5 of the series of plates in amounts of 10, 5, 2, and 1 drops, respectively. The liquid in the center of the plates was spread over the surface with a glass rod, about 5 mm. in diameter and 18 cm. long, and bent at a point 9.5 cm. distant from 1 end so that the 2 ends of the rod were parallel and a few millimeters apart. The other end of the rod at a point immediately above the center of the 9.5 cm. length was made at a right angle and attached to a piece of thin-wall, Para rubber tubing of suitable diameter, and 3-4 cm. in length. In the other end of the rubber tubing was inserted a piece of glass rod 6-8 cm. long, which served as a handle. To obtain flexibility, a distance of 0.5-1 cm. was allowed between the ends of the glass rods inside the tubing. An ordinary T tube will serve the purpose equally well by cutting and sealing the ends of the long surface at a proper length to fit loosely inside the bottom of a Petri dish and attaching the handle and rubber to the remaining end.

In spreading a series of plates by this method, the spreader is brought in contact with the medium and fluid of the 1st plate of the series, and with the handle in a perpendicular position, is rotated through 180°. It is then transferred directly to the 2nd plate, and the process repeated, and so on through the series of plates. As the last 1-3 of the series of plates contain none of the fluid, their inoculation is made by carrying over decreasing amounts from the previous plates with the spreader. This method has an advantage over the ordinary L-rod, in that the distribution of the colonies over the surface of the plate is

more even, and because of its flexibility, the pressure is uniform on all parts of the plate and the medium is not marred.

Sterilization is best effected by autoclaving several of the spreaders in a suitable container, preferably a metal box with the top partly removed.

In each test, a control series of plates was made with a known culture of *B. typhosus*. After 18-24 hours' incubation at 37 C., the plates were examined and a number of typhoid-like colonies picked and transferred together with the typhoid control to Russell's medium. In examining the plates for typhoid colonies, I found it advantageous to compare the typhoid-like colonies with those on the typhoid-control plate, having approximately the same number of colonies as the total number on the plate under examination, since the typhoid colonies differ in appearance on heavily and lightly seeded plates.

After 24 hours' incubation at 37 C., the cultures on Russell's medium were examined. If the characteristic reaction was obtained in any of the tubes, the surface growth was washed off with sterile salt solution, the suspension filtered, and a macroscopic agglutination test made with antityphoid serum. If agglutination occurred, the culture was replated and further tested as to its action in lactose, dextrose, and dextrin broths, gelatin, neutral red, and peptone (peptone tested after 7 days for indol-production). Identification was considered complete in case the culture was agglutinated in a dilution of 1:1000 of serum, and gave characteristic reactions on the media referred to above.

In case none of the colonies picked from the Endo plates on 1st examination gave the characteristic reaction in Russell's medium, the plates were again examined and more colonies transferred. In some instances in this 2nd, or 48-hour examination of the Endo plates, *B. typhosus* was isolated after failure in the 1st 18- or 24-hour examination. Two or 3 consecutive, negative tests were made from the plants before an experiment was complete. In each test, not less than 30-50 Endo plates were made and a like number of colonies transferred to Russell's medium.

In cases where the longevity of the typhoid bacillus was determined in the soil, about 1 gm. of the soil was used and added to 1 liter of sterile water in an Erlenmeyer flask and shaken; small amounts of the mixture were then transferred with sterile pipettes to Endo plates and examination made by the same method as was used for the vegetables.

RESULTS OF INDOOR EXPERIMENTS

Exper. 1.—Lettuce was grown in sandy soil which was inoculated, before the plants appeared above the surface, with Strain 1, by adding the surface growth from 5 agar slants, after 24 hours' incubation at 37 C., suspended in one-half liter of physiologic salt solution.

TABLE 1
RESULTS OF TESTS ON LETTUCE GROWN IN SOIL INOCULATED WITH *B. TYPHOSUS* (STRAIN 1)

Date Planted	Date Inoculated	Material Examined	Date of Last Positive Examination	Length of Time after Inoculation (days)
Dec. 2	Dec. 5	Washed leaves and stems after maceration in water	Dec. 23	18
	Dec. 5	Soil	Jan. 27	53

In 2 later tests, one Dec. 28 and the other on Jan. 4, *B. typhosus* was recovered from the rinse water.

Exper. 2.—Radishes were grown in garden soil to which had previously been added a small amount of fine manure. Inoculation of the soil was made with Strain 1, using 10 c.c. of a 24-hour broth culture, diluted with 500 c.c. of sterile dilute sewage.

TABLE 2
RESULTS OF TESTS ON RADISHES GROWN IN SOIL INOCULATED WITH *B. TYPHOSUS* (STRAIN 1)

Date Planted	Date Inoculated	Material Examined	Date of Last Positive Examination	Length of Time after Inoculation (days)
Dec. 2	Dec. 5	Washed roots and stems	Jan. 27	53
	Dec. 5	Soil	Feb. 17	74

Negative results were obtained in 1 examination from the washed plants on Jan. 13. On Jan. 27, the organism was not recovered from the rinse water, but was isolated from the 1st rinse water in a test on Feb. 2. In an examination from the soil on Feb. 13, negative results were obtained.

Exper. 3.—The soil used in this experiment was from the same source as that used in *Exper. 2*. Radish seeds were sown on April 3 without further enrichment. Inoculation was made with Strain 2, which had been isolated from a fresh typhoid stool on March 15, and had been transferred 5 times on agar. The surface growth from a one-liter flask, after 24 hours' incubation at 37 C. and suspended in 500 c.c. of tap water, was added to the surface of the soil.

TABLE 3
RESULTS OF TESTS ON RADISHES GROWN IN SOIL INOCULATED WITH *B. TYPHOSUS* (STRAIN 2)

Date Planted	Date Inoculated	Material Examined	Date of Last Positive Examination	Length of Time after Inoculation (days)
Apr. 3	Apr. 5	Washed roots and stems	May 10	35
	Apr. 5	Soil	May 24	49

In this case the 1st test could not be made until May 6. At this time, a positive result was obtained with the washed roots and stems. A 2nd test on May 8 was negative. On May 23, negative results were obtained from the soil.

OUTDOOR EXPERIMENTS

Experiments were begun in the open in May, 1915, and continued through the growing season. A plot of ground was selected, which afforded ideal conditions in regard to natural outdoor conditions, such as exposure to sunshine, rainfall, and atmospheric changes. Two beds, 4 by 6 feet were prepared by removing the soil to a depth of 12 inches, and filling in with garden soil, then adding a small amount of fine

manure. The soil was prepared for seeding in a manner similar to that used under ordinary conditions. The beds were screened to prevent the danger of chance spread of infection by insects. The seeds were planted in rows of ordinary width. After the plants appeared above the surface of the soil they were cultivated at intervals 7-10 days.

Typhoid stools were used for inoculation; in each case from the feces used, Endo plates were made and examined in the usual manner, and only those samples which showed the presence of *B. typhosus* in considerable numbers were used. In case the colonies picked from the Endo plates gave the characteristic reaction in Russell's medium but failed to show agglutination with antityphoid serum, the sample was discarded. In 2 samples this was found; however, in tests made after the culture had been transferred several times on agar, both showed agglutination.

Expér. 4.—Radish and lettuce seeds were planted in one of the beds on May 19. On the 4th day after planting, and before the vegetables were above the surface, the soil was inoculated. One c.c. of a mixture of fresh typhoid feces and urine was distributed on the surface of 3 one-liter agar flasks, and incubated for 18 hours at 37 C. The growth was washed off and added to the original stool. This mixture was then diluted in about 6 liters of water and sprinkled on the soil with an ordinary garden sprinkler, after which time the soil received no further water except by natural precipitation. The examinations were made in the manner described, with the exception that in this and succeeding experiments, an examination of the rinse water from the vegetables was not made, but larger amounts of the fluid from the macerated plants after washing was plated out.

TABLE 4
RESULTS OF TESTS ON LETTUCE AND RADISHES GROWN IN SOIL INOCULATED WITH *B. TYPHOSUS*
FOUR DAYS AFTER PLANTING

Date Planted	Date Inoculated	Material Examined	Date of Last Positive Examination	Length of Time after Inoculation (days)
May 19	May 23	Washed leaves and stems of lettuce	June 13	21
May 19	May 23	Washed roots and stems of radishes	June 29	37
	May 23	Soil	July 3	41

The vegetables in each case were removed from the beds 2 days prior to testing and allowed to remain in the laboratory.

From May 23 to June 13, the respective dates of soil inoculation and removal of the lettuce previous to the last positive test, there were 138.9 hours of sunshine, a total precipitation of 3.47 in., with an average relative humidity of 73.9%. The mean temperature during the period was 57.7 F. From May 23 to June 27, the respective dates of soil inoculation and removal of the radishes previous to the last testing, there were 276.2 hours of sunshine, 6.21 in. of rainfall, a mean temperature of 60.7 F. and a mean relative humidity of 70.8%.

Exper. 5.—Radish seeds were planted July 5, in a bed to which fresh typhoid stools had been added 4 days previously. Approximately 1 liter of excreta was used, which was at once diluted with about 6 liters of water and added directly to the soil. Two days previous to each test, the radishes were removed from the soil and exposed to laboratory conditions.

TABLE 5
RESULTS OF TESTS ON RADISHES GROWN IN SOIL INOCULATED WITH *B. TYPHOSUS*
FOUR DAYS PREVIOUS TO PLANTING

Date Planted	Date Inoculated	Material Examined	Date of Last Positive Examination	Length of Time after Inoculation (days)
July 5	July 1	Washed roots and stems	July 29	28
	July 1	Soil	Aug. 4	34

From July 1 to July 27, there was a total of 211 hours of sunshine, 4.1 in. of rainfall, and a mean relative humidity of 66.16%. The mean temperature was 70 F. Negative results were obtained on July 23 and July 26, in examination of the radishes.

Exper. 6.—Radish seeds were planted on July 17 and the soil inoculated in the same manner as in *Exper. 4*.

TABLE 6
RESULTS OF TESTS ON RADISHES GROWN IN SOIL INOCULATED WITH *B. TYPHOSUS*

Date Planted	Date Inoculated	Material Examined	Date of Last Positive Examination	Length of Time after Inoculation (days)
July 17	July 17	Washed roots and stems	Aug. 21	35
	July 17	Soil	Aug. 21*	35

* No further tests were made on the soil after this date.

In this experiment, the vegetables to be tested were removed to the laboratory 3 days previous to the testing.

The total hours of sunshine was 206.7; precipitation, 5.65 in.; mean relative humidity, 77%; and mean temperature, 70.5 F.

In *Expers. 4, 5, and 6*, the vegetables at about 20-25 days after planting were equal in size to those ordinarily found in the markets.

The possibility of *B. typhosus* entering the interior of the plant, through injury to the roots during cultivation, suggested itself. To determine if such might occur, radishes which were about one-half matured in soil contained in pots which had not been inoculated with *B. typhosus* were used. The small root tips were injured by crushing, the tops of the pots were covered with heavy paraffined paper and sealed on the edges. The tops of the radishes were allowed to protrude through holes cut in the paper. The stems, at the point of passage through the paper were carefully wrapped with cotton. By this means no communication of the bacteria could take place from the soil to the top of the plant, except through the stems. A suspension of *B. typhosus* was then added to the soil through a glass tube, entering below the paraffined paper. The tests consisted in examining the leaves and upper stems at periods of 2-3 days for the typhoid organism, by the method used in the other experiments. In no cases were results obtained which would indicate a transference of the organism through the interior of the plant.

As a further test, radishes which had been taken from an infected bed were seared over the surfaces. They were then cut in halves, and the cut surfaces rubbed over Endo plates. The parts were then macerated in physiologic salt solution and the mixture planted. In no instance was the organism found in the interior of the plants.

Experiments were made in the autumn of 1916 for the purpose of comparing the longevity of old and freshly isolated strains of *B. typhosus* in both sandy and garden soils.

The results are shown in Table 7. Strain 3 has been used as a laboratory culture for several years. Strains 4 and 5 were isolated from the fresh stools of typhoid patients and used 2 and 5 days, respectively, after isolation.

The sandy soil was quite dry at the time of the inoculation and contained a small amount of organic matter. The garden soil was approximately the same, and prepared similar to that previously described in the outdoor experiments.

The soils were seeded Sept. 4, using for each plot (4 by 6 ft.) the 24-hour surface growth from 3 one-liter, slant agar flasks, which was diluted with 6 liters of water and sprinkled on the surface, after which the soil received no further addition of moisture except that of natural precipitation.

Tests were made by the previously described method, using a mixture of soil from the surface to a depth of 5 in.

TABLE 7
RESULTS OF TESTS COMPARING THE LONGEVITY OF OLD AND FRESH STRAINS OF *B. TYPHOSUS*
IN SANDY AND GARDEN SOILS

Soil	Strain of <i>B. Typhosus</i>	Date of Last Positive Exam.	Length of Time after Inoculation (days)	Hours of Sunshine	Total Precipitation (in.)	Mean Temp. (F.)
Sandy	3	Oct. 10	36	312.8	2.15	63.1
Sandy	4	Oct. 3	29	248.6	1.77	61.5
Garden	3	Nov. 1	58	441.3	3.6	57.5
Garden	1	Oct. 24	50	384.4	2.59	58.5
Garden	4	Oct. 6	32	272.9	1.77	62.0
Garden	5	Oct. 17	43	361.8	2.58	60.4

DISCUSSION

It is a comparatively easy matter in the beginning of such experiments here reported to recover the typhoid bacilli; but in the latter part, as the specific organisms decrease in numbers, their isolation becomes quite difficult. There is present at this time a resistant minority which persists after the greater number have disappeared. The number is small in comparison with the total bacterial flora, but it would be unreasonable to conclude that even after several negative tests, typhoid bacilli might not be present in sufficient numbers to cause infection. For this reason, too definite conclusions cannot be drawn as to the longevity of *B. typhosus* in soils. However, the marked differences in the viability which have been shown by the old and fresh cultures, particularly, in Exper. 7, indicate that long-continued growth outside the human body tends to add to their resistance in soil. The lesser differences in cases of fresh cultures also suggest considerable variation even with freshly isolated strains. This point may explain, in part, the discrepancies in the reports of many workers on the viability of *B. typhosus* in soil.

The extreme tenacity with which the typhoid organisms adhere to the surface of the plants grown in infected soils is shown in these experiments. The methods of washing the vegetables have probably been as thorough as those employed ordinarily in the preparation of such foods for table use. In but few instances has washing freed the plants of the bacilli. For this reason, vegetables from infected soil may be regarded unsafe, as long as typhoid bacilli survive in the soil.

The temperature throughout this work has shown no great variation; therefore, no conclusions have been reached relative to this factor. Determination of the longevity in frozen soils has not been undertaken.

In the outdoor tests, the conditions in Exper. 5 were identical with those which might occur naturally. Experiments 4 and 6 differed only in that they received heavier inoculations. In each of these experiments, *B. typhosus* was isolated from the plants after they had reached maturity. These vegetables had been under conditions identical, so far as possible, with those obtained in natural growing, marketing, and cleaning of such foods.

It is not only important to examine the leaves and stems in such experiments, but also to examine the root where it is used for food. The root is in contact with the infected soil and protected from sun-

shine and other conditions unfavorable to the viability of the organism and consequently is more likely to transmit infection. This has been shown in Expts. 1 and 4.

A brief attempt was made to determine the extent to which the manuring of soils with human excreta is carried on in this country. Letters of inquiry requesting such information were sent to health officers and others connected with public health work in various localities. Replies from the majority conveyed but little definite information. In 2 localities, it was found that this practice was in vogue to some extent. It is probably not so uncommon as is generally supposed, especially in suburban, village, and rural districts, where the methods of sewage disposal are more or less inadequate.

SUMMARY AND CONCLUSIONS

In the work herein reported, the longevity of *B. typhosus* in soils has shown considerable variation, under like conditions in the open. The old strains (1 and 3) survived in garden soil 50 and 58 days, while the viability of fresh cultures (Strains 4 and 5) was 32 and 43 days, respectively.

In sandy soil, the longevity of Strain 3 was 36 days; that of Strain 4, 29 days.

In 3 outdoor experiments, extending from May to September, *B. typhosus* was isolated from garden soil inoculated with typhoid excreta after 41, 34, and 35 days.

Under hot-house conditions in sandy soil, Strain 1 survived 53 days. The longevity was increased under the same conditions in garden soil enriched with sterile sewage and broth to 74 days. Similarly, the viability of fresh culture of Strain 2 in garden soil was 49 days.

No evidence has been found that would indicate the entrance of *B. typhosus* into the interior of the plants.

The organisms became attached to the surfaces from their contact with the soil and are not removed by ordinary washing.

Under natural conditions, radishes grown in contaminated soil were found to be still infected with typhoid bacilli in 3 experiments after periods of 37, 28, and 35 days, respectively; and from lettuce, after 21 days. This is ample time for the maturing of such vegetables.

It may be reasonably concluded that vegetables grown in soil fertilized with fresh typhoid excreta shortly before planting or during the

growing season are likely to be contaminated at the time they reach the consumer.

Vegetables so contaminated are not made safe by the ordinary methods used in the preparation of such foods for table use, and may, therefore, be a source of typhoid infection.